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PCB, DDT, arsenic, and heavy metal (Cd, Cu, Pb, and Zn) concentrations in chameleon (*Chamaeleo chamaeleon*) eggs from Southwest Spain

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Abstract

This work presents the concentrations of twenty PCB congeners, *p,p′*-DDT, and its two main metabolites, *p,p′*-DDE, *p,p′*-TDE, As, Cd, Cu, Pb, and Zn found in common chameleon eggs from nine different nests located in Southwest Spain. Of the heavy metals and arsenic, Zn and Cu exhibited the highest concentrations in egg contents (ranging from 10100 to 12950 and from 567 to 706 ng g^{-1} wet weight basis [w.w.], respectively) and eggshells (ranging from 5605 to 13290 ng g^{-1} w.w. for Zn and from 1487 to 4361 ng g^{-1} w.w. for Cu). Total PCB concentrations in egg contents ranged from 32 to 52 ng g^{-1} w.w. and were higher than total dichlorodiphenylethanes concentrations (ranging from 0.67 to 1.9 ng g^{-1} w.w., calculated as the sum of *p,p′*-DDT plus *p,p′*-DDE and *p,p′*-TDE). Comparison of the data from the present study with the data from a study conducted in 1997 revealed a large decrease in Pb concentration and a twofold increase in PCB concentrations. Taking into account all the pollutants investigated, the contamination level found in common chameleon eggs from Southwest Spain was generally lower than has been reported in the literature for eggs of different reptile species. However, it should be borne in mind that most of the data found in the literature refer to highly polluted areas.

Keywords: PCBs; DDTs; Heavy metal; Arsenic; Common chameleon eggs

1. Introduction

Reptiles are presently considered susceptible to a number of factors which have contributed to the global decline of several reptile species like turtles, crocodilians or lizards (Gibbons et al., 2000). Environmental pollution is one of the main threats affecting the conservation of reptile populations, and some reptile species have been identified as good bioindicators of pollution in their environments (Lambert, 1997; Loumbourdis, 1997; Crain and Guillete, 1998). Organochlorine compounds, such as polychlorinated biphenyls (PCBs) and dichlorodiphenyl ethanes (DDTs), are notoriously toxic and persistent contaminants which accumulate in food chains and are associated with habitat and dietary habits. Concern over levels of organochlorine compounds and their estrogenic effects has been increasing in recent years. In many oviparous species, PCB pollution can produce severe impacts on eggs, leading to embryo death or abnormal embryonic development (Gibbons et al., 2000). As for heavy metals and arsenic, although there is little information in the literature about the accumulation and effects of metals in reptiles, some studies have demonstrated that reptiles accumulate high concentrations of metals (Hopkins et al., 2002). In addition, eggs of many oviparous species have permeable shells which may absorb contaminants from the soil, mainly in polluted areas (Brewer et al., 1999; Marco et al., 2004).

The common chameleon (*Chamaeleo chamaeleon*) is widely distributed in North Africa, while in Europe its distribution is circumscribed to the Southwest of the Iberian

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Peninsula and isolated areas in circum-Mediterranean countries. In Spain, chameleons are frequently present in urbanized and cultivated areas (Cuadrado, 2002). In a previous study carried out by Díaz-Paniagua et al. (2002) the presence of organic and inorganic contaminants in chameleon eggs was detected. In particular, high concentrations of lead and PCBs were found as compared to the other elements investigated and DDTs and were associated with the influence of industrial or urban sources such as vehicle exhausts or industrial residues.

This paper is a continuation and extension of that earlier study on the presence of organic and inorganic contaminants in the common chameleon. The two studies are the first to present data on environmental pollutants in chameleon egg contents and shells. The paper presents the concentrations of twenty PCB congeners, *p*,*p*′-DDT (2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane) and its two main metabolites, *p*,*p*′-DDE (2,2-bis(4-chlorophenyl)-1,1-dichloroethylene) and *p*,*p*′-TDE (2,2-bis-(4-chlorophenyl)-1,1-dichloroethane), heavy metals (Cd, Cu, Pb, and Zn) and arsenic in common chameleon eggs collected in 2001 from Southwest Spain. These results are also compared with the findings for eggs of that particular species collected in the same area in 1997.

2. Materials and methods

2.1. Sample collection

Chameleon eggs were collected from 0 to 15 days after laying from nine natural chameleon nests at different coastal locations in the province of Cádiz (Southwest Spain) in 2001. Sample clutches numbers 1–4 were from the area around Rota, sample clutches 5, 7, and 8 from the area around Puerto de Santa María and sample clutches 6 and 9 from the area around Puerto Real (see Fig. 1). Two eggs from sampling point 6 and one egg from point 8 exhibited significantly more loss of water than the other eggs and were discarded. Egg contents, yolk and white, from the same sampling point were pooled, freeze-dried, and stored at room temperature before extraction and analysis.

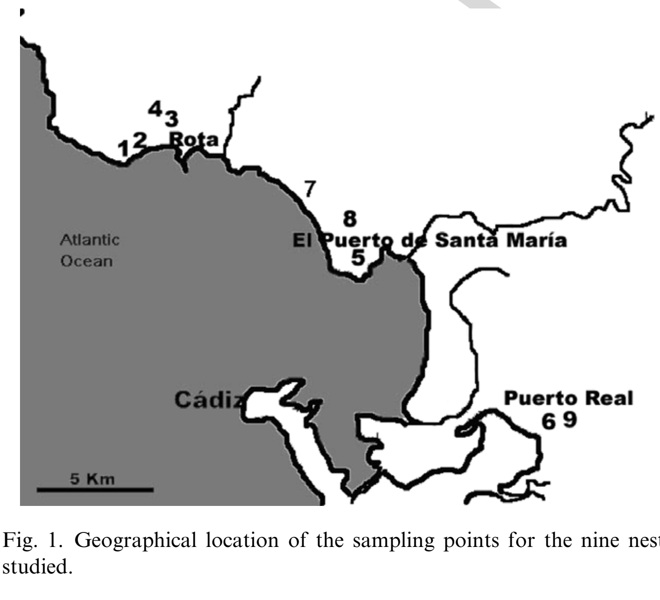

Fig. 1. Geographical location of the sampling points for the nine nests studied.

2.2. Chemicals

The trace elements chosen for the present study were As, Cd, Cu, Pb, and Zn. They were selected because they were known to be present in the surrounding area due to pyrite mining near the province of Cádiz. The following organochlorinated compounds were measured: *p*,*p*′-DDT, *p*,*p*′-DDE, *p*,*p*′-TDE; and PCBs 28, 52, 95, 101, 105, 114, 118, 123, 132, 138, 149, 153, 156, 157, 167, 170, 180, 183, 189, and 194 (Ehrenstorfer, Augsburg, Germany) according to Ballschmitter and Zell, 1980. These congeners were selected because of their toxicity and relative abundance in both technical mixtures and environmental samples (Frame et al., 1996; van den Berg et al., 1998). 1,2,3,4-Tetrachloronaphthalene (TCN) and PCB 209 were used as injection standards for PCB and DDT quantification. Hexane (Unisolv quality), silica gel 60 (0.063–0.2 mm particle size) and sulphuric acid used for PCB and DDT sample preparation were from Merck (Darmstadt, Germany), and anhydrous sodium sulphate was from J.T. Baker (Deventer, The Netherlands). Hydrogen peroxide and nitric acid employed for trace element sample preparation were from Panreac (Barcelona, Spain) and Merck, respectively.

2.3. PCBs and DDTs: analytical procedure

For sample preparation, a miniaturized one-step method previously validated for the determination of PCBs in fatty foodstuffs (Ramos et al., 2004) was used to determine PCBs and DDTs in chameleon eggs. The original method, involving 0.1 g of sample, was adapted to the amount of sample available for each nest. Briefly, the methodology consisted in spreading the freeze-dried egg samples (between 0.4 and 1.0 g) on similar amounts of Na_2SO_4 and silica modified with 44% (w/w) sulphuric acid (SiO_2/H_2SO_4). After blending and homogenizing, the mixture was packed in a disposable glass extraction column on top of neutral silica (one and a half times the sample amount) and SiO_2/H_2SO_4 (three times the sample amount). A maximum of 50 ml of hexane was used as extracting solvent. After two 10 min static extractions, some fresh solvent was eluted through the column to ensure proper purging of the sample and clean-up of the sorbent. Blanks were prepared following the same procedure as for eggs but without sample. No background interferences were detected by the analytical instruments used for the final determination.

Instrumental determinations were performed by gas chromatography coupled with an Agilent 6890 Series II GC-micro-ECD (Agilent Technologies, Palo Alto, CA, USA) as described elsewhere (Gómara et al., 2002). Samples were injected in hot splitless mode (1 μl, 270 °C, splitless time 1.0 min) into a DB-5 capillary column (J&W Scientific, USA; 60 m, i.d. 0.25 mm, film thickness 0.25 μm). The column temperature was programmed from 80 °C (2 min) to 185 °C (3 min) at a rate of 30 °C min^{-1}, then to 230 °C (10 min) at 1.5 °C min^{-1} and then to 270 °C (10 min) at 5 °C min^{-1}. Nitrogen was used as carrier gas (constant flow, 1.5 ml min^{-1}) and as make-up gas (30 ml min^{-1}). The detector temperature was set at 300 °C. The relative standard deviations (RSDs, $n = 3$) for repeatability and reproducibility were always lower than 10% and the detection limits (LODs) were between 0.009 and 0.1 ng g^{-1} of freeze-dried sample. Results for blank samples were lower than the LODs in all cases; a representative chromatogram of one of the chameleon egg samples is shown in Fig. 2. Moreover, the working group participated in several international quality control studies for the measurement of PCBs and other persistent organic pollutants (POPs) in different biological and food matrices, such as "Interlaboratory Comparison on Dioxins in Food" from 2000 to 2005, organised by National Institute of Public Health, "NIST/NOAA Inter-laboratory Comparison Exercise Program for Organic Contaminants in Marine Mammal Tissue" in 2003, and "AMAP Interlaboratory Exercise: Ring Test for PCBs, PBDEs and OCs" from 2002 to 2005, organised by National Institute of Public Health, Quebec, Canada. The results were consistent at all times with the consensus means given by the inter-laboratory organizations.

2.4. Heavy metals and arsenic: analytical procedure

Approximately 0.1 g of pooled freeze-dried egg samples were digested using an acid medium according to a methodology published previously (Hernández et al., 1999). Measurements of copper and zinc were performed using a flame atomic absorption spectrometer (AAS) (Spectra A-100; Varian, Iberica, Madrid, Spain). Cadmium, lead, and arsenic were measured using a Perkin Elmer (Hispania, Madrid, Spain) longitudinal AC Zeeman AAS (AAnalyst 600) equipped with a transversely heated graphite atomizer. The limits of quantification (LOQs) were: arsenic 0.4 ng g^{-1} (expressed on a wet weight basis, w.w.), cadmium 0.16 ng g^{-1} w.w., copper 0.06 μg g^{-1} w.w., lead 1.2 ng g^{-1} w.w., and zinc 0.2 μg g^{-1} w.w.. All specimens were analysed in batches with method blanks, known standards and reference materials. Accepted recoveries of reference materials ranged from 88% to 110%. Relative standard deviations (RSDs) in replicates and reference materials were lower than 10% in all cases.

3. Results and discussions

Table 1 shows the PCB and DDT concentrations (expressed on a wet weight basis, w.w.) found in the contents of chameleon eggs collected from the nine different nests in southern Spain. Overall, the twenty PCB congeners determined presented no variations in respect of sampling sites (sampling points 1 to 5, 7, and 8 in the Rota and Puerto de Santa María areas and sampling point 9 in Puerto Real). One of the clutches from Puerto Real (sampling point 6) exhibited the highest PCB values. Total PCB concentrations ranged from 32 to 52 ng g^{-1} w.w. The highest value was found in nest number 6 and the lowest in nest number 2. For comparison of these results to previous data from the same area, total PCB concentrations were re-calculated as the sum of the 12 congeners determined in the previous study in 1997 (Díaz-Paniagua et al., 2002). These values (25–40 ng g^{-1} w.w.) were higher than in eggs from the same species in the same locations in 1997, which ranged from 2.9 to 33 ng g^{-1} w.w. (Díaz-Paniagua et al., 2002). The increase of PCB concentrations in chameleon egg contents could be due to the different sampling periods.

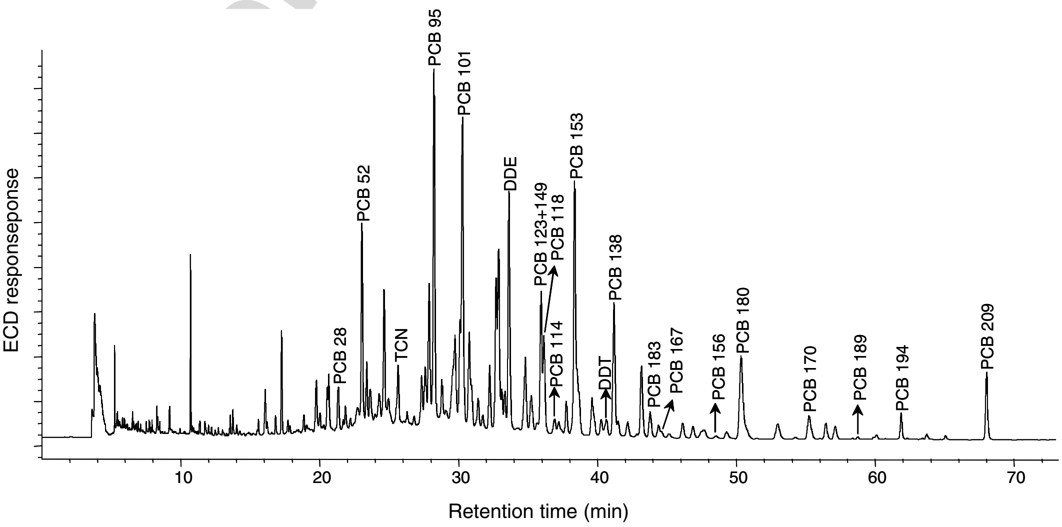

Fig. 2. Chromatogram of a chameleon egg sample.

Table 1
PCB and DDT concentrations (ng g^{-1} on wet weight) in chameleon egg clutches collected in Southwest Spain in 2001 and total concentrations corresponding to 1997 samples

PCB no.[a]	Clutch 1 ($n=4$)[b]	Clutch 2 ($n=4$)[b]	Clutch 3 ($n=4$)[b]	Clutch 4 ($n=4$)[b]	ROTA – mean (SD)	Clutch 5 ($n=4$)[b]	Clutch 7 ($n=4$)[b]	Clutch 8 ($n=3$)[b]	S. MARIA – mean (SD)	Clutch 6 ($n=2$)[b]	Clutch 9 ($n=4$)[b]	P. REAL – mean (SD)
28	5.9	5.8	6.2	6.3	6.1 (0.2)	5.9	6.9	6.2	6.3 (0.5)	5.4	5.3	5.4 (0.1)
52	9.0	8.4	9.4	10	9.2 (0.7)	8.1	11	11	10 (1.7)	12	7.4	9.7 (3.3)
95	6.0	5.9	6.8	7.1	6.5 (0.6)	5.8	7.6	8.4	7.3 (1.3)	9.3	5.3	7.3 (2.8)
101	5.5	5.3	6.4	6.7	6.0 (0.7)	5.3	7.3	8.1	6.9 (1.5)	9.4	4.9	7.1 (3.1)
123 + 149	0.88	0.93	0.98	0.96	0.94 (0.05)	0.96	1.0	1.3	1.1 (0.2)	1.7	0.79	1.2 (0.6)
118	0.63	0.66	0.72	0.69	0.68 (0.04)	0.76	1.9	0.99	1.2 (0.6)	1.3	0.94	1.1 (0.3)
114	0.01	0.02	0.04	0.03	0.02 (0.01)	0.02	0.06	0.02	0.03 (0.02)	0.03	0.03	0.03 (–)
153	2.8	2.5	2.8	3.2	2.8 (0.3)	2.7	4.5	3.3	3.5 (0.9)	5.5	6.3	5.9 (0.5)
132	0.27	0.24	0.26	0.26	0.25 (0.01)	0.21	0.21	0.32	0.25 (0.06)	0.46	0.14	0.30 (0.22)
105	0.17	0.18	0.19	0.17	0.18 (0.01)	0.26	0.31	0.24	0.27 (0.03)	0.54	0.16	0.35 (0.27)
138	1.2	1.1	1.4	1.6	1.3 (0.2)	1.1	1.8	1.4	1.5 (0.4)	2.8	1.9	2.4 (0.7)
183	0.08	0.08	0.07	0.09	0.08 (0.01)	0.11	0.08	0.05	0.08 (0.03)	0.14	0.14	0.14 (–)
167	0.01	0.01	0.01	0.01	0.01 (–)	0.03	0.04	<0.02	0.02 (0.02)	0.02	0.06	0.04 (0.03)
156	0.03	0.03	0.03	0.03	0.03 (–)	0.06	0.06	0.03	0.05 (0.02)	0.09	0.08	0.09 (0.01)
157	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
180	0.64	0.61	0.51	1.3	0.75 (0.34)	1.4	0.71	0.55	0.88 (0.44)	1.4	3.2	2.3 (1.2)
170	0.33	0.30	0.29	0.43	0.34 (0.06)	0.56	0.32	0.33	0.40 (0.14)	0.82	0.85	0.84 (0.02)
189	0.04	0.04	0.03	0.03	0.04 (–)	0.05	0.04	0.04	0.05 (0.01)	0.08	0.07	0.07 (0.01)
194	0.35	0.34	0.22	0.31	0.30 (0.06)	0.52	0.30	0.30	0.37 (0.13)	0.57	0.96	0.77 (0.27)
$\sum_{20}$ PCBs[c]	34	32	36	39	35 (3)	34	44	43	40 (8)	52	39	45 (13)
$\sum_{12}$ PCBs (2001)[d]	27	25	28	31	28 (3)	27	5	32	31 (6)	40	32	36 (9)
$\sum_{12}$ PCBs (1997)	9.2	34	8.3	26	19 (12)	16	4.6	2.9	7.8 (6.9)	19	33	26 (5)
p,p'-DDE	0.57	0.61	0.68	0.63	0.62 (0.04)	0.48	0.76	0.68	0.64 (0.15)	1.5	0.53	1.0 (0.7)
p,p'-TDE	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
p,p'-DDT	0.18	0.22	0.20	0.20	0.20 (0.01)	0.19	0.28	0.25	0.24 (0.04)	0.37	0.15	0.27 (0.16)
$\sum$DDTs (2001)	0.75	0.83	0.88	0.83	0.82 (0.06)	0.67	1.0	0.94	0.88 (0.19)	1.9	0.68	1.3 (0.8)
$\sum$DDTs (1997)	0.34	1.7	0.26	1.3	0.89 (0.71)	0.80	0.17	0.12	0.36 (0.37)	1.7	1.7	1.7 (–)

Mean and standard deviation corresponding to the tree different areas investigated.
[a] PCB no. according to Ballschmitter and Zell (1980).
[b] Number of samples pooled for each nest.
[c] Calculated as the sum of the twenty congeners selected in the present study (2001).
[d] Calculated as the sum of the 12 congeners determined in the previous study in 1997.

PCBs 28, 52, 95, and 101 were the most abundant of the PCB congeners investigated, accounting for 11–27% of the total PCBs investigated. These were followed by PCBs 153 (8–16%), 138 (3–5%) and 180 (1–8%), except in eggs collected at sampling site 9, where PCBs 153 and 180 were the most abundant (Fig. 3). The PCB pattern in biological samples mainly reflects the PCB contamination in the area and the species capacity of PCB biodegradation and bioaccumulation. Differences in PCB patterns from the same species (e.g. audouin gulls and snapping turtles) living in different areas have been reported in the literature (González et al., 1991; Dabrowska et al., 2006). Then again, PCB patterns dominated by the lower chlorinated PCBs have been found in animals in the lowest trophic levels, such as groupers and turtles (Serrano et al., 2000; Dabrowska et al., 2006).

Although detectable PCB concentrations have been found in chameleon egg contents, pollution levels are far below those reported for snapping turtle eggs from well-known contaminated areas like Akwesasne, Canada (de Solla et al., 2001) and several sampling sites at the Great Lakes (Canada) from 1989 to 1991 (Bishop et al., 1998). Only one sampling point of snapping turtle eggs from the Great Lakes presents PCB concentrations lower than the common chameleon egg concentrations found in this study. This fact indicates that the area studied in the present work is not a highly polluted one like the Great Lakes. Total PCB concentrations found in chameleon eggs in this study were similar to or slightly higher than those found in loggerhead and green sea turtle eggs in the Mediterranean Sea (Mackenzie et al., 1999) and in Merrit Island (Florida, USA) (Clark and Krynitsky, 1985).

Concentrations of DDTs were comparable at all sampling sites, ranging from 0.67 to 1.0 ng g^{-1} w.w., except for clutch number 6 (1.9 ng g^{-1} w.w.) (Table 1). These values were similar to those found in chameleon eggs collected in 1997 in the same area, in the range 0.12–1.7 ng g^{-1} w.w. (Díaz-Paniagua et al., 2002). *p,p′*-DDT and its main metabolite *p,p′*-DDE were detected in all egg samples analysed, while the metabolite *p,p′*-TDE was only detected in sample from nest number 7. The ratio *p,p′*-DDE/*p,p′*-DDT (>1) at all sampling points indicated that *p,p′*-DDT had not been used in this area in last few years.

As in the case of PCBs, DDT levels found in common chameleon eggs from Southwest Spain are lower than in snapping turtle eggs from Canada (Bishop et al., 1998; de Solla et al., 2001).

Heavy metal and arsenic concentrations (expressed in ng g^{-1} on w.w.) in egg contents and eggshells are shown in Table 2. Arsenic concentrations found in egg contents were the lowest, followed by Cd, Pb, Cu, and Zn. All were similar in the nine clutches investigated, except for Pb, concentrations of which ranged from ND to 35 ng g^{-1} (w.w.). In the case of eggshells, Cd presented the lowest concentrations, followed by As, Pb, Cu, and Zn. All clutches presented similar eggshell concentrations for As, Cu, and Zn; however, nests numbers 4 and 5 contained high Cd concentrations (235 and 120 ng g^{-1} w.w., respectively), and clutches 3 and 4 contained high Pb concentrations (1454 and 1005 ng g^{-1} w.w., respectively). Cd, Cu, and Zn concentrations in egg contents were similar in samples from 1997 (Díaz-Paniagua et al., 2002) and 2001, but Pb concentrations decreased from 1997, when they ranged from 4000 to 22 100 ng g^{-1} w.w. (Díaz-Paniagua et al., 2002), to 2001 (<0.02–35 ng g^{-1} w.w.). This could be connected with a general decline in the use of Pb, for instance as an additive in petrol and paint (Thomas et al., 1999). It is important to note that the concentration of Pb was much higher in eggshells (ranging from 185 to 1454 ng g^{-1} w.w.) than in egg contents (<1.2–35 ng g^{-1} w.w.). The same trend was observed for As, Cd, and Cu while the opposite was observed for Zn, concentrations of which were greater in egg contents than in eggshell.

Although there is little information about metal concentrations in reptile species, some comparison was possible. Concentrations of Cd, Pb, and As were generally lower

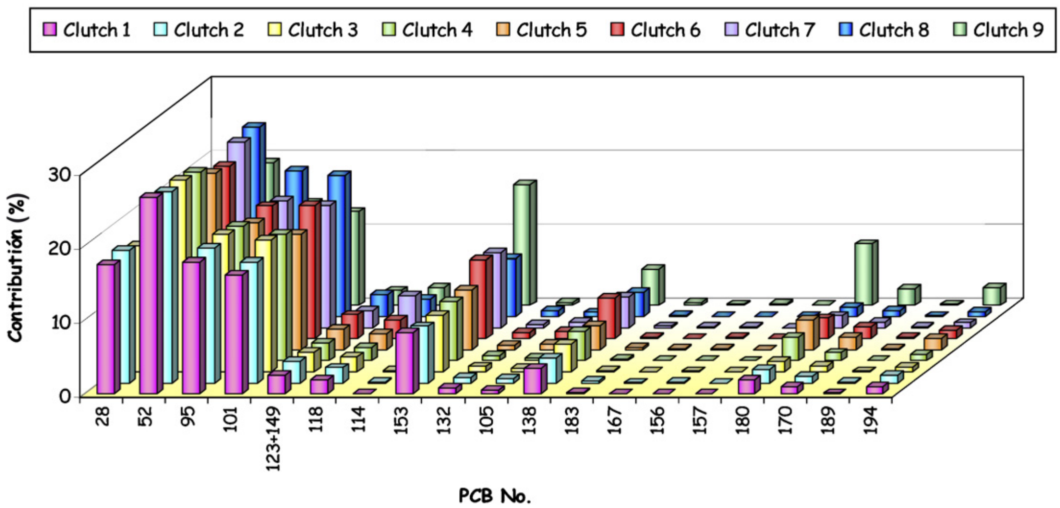

Fig. 3. Percentage contribution of PCB congeners to the total PCB concentrations for each clutch investigated.

Table 2
Arsenic and heavy metal concentrations (ng g^{-1} on wet weight) in chameleon egg contents and eggshells from clutches collected in Southwest Spain in 2001

	Clutch 1 ($n = 4$)[a]	Clutch 2 ($n = 4$)[a]	Clutch 3 ($n = 4$)[a]	Clutch 4 ($n = 4$)[a]	ROTA – mean (SD)	Clutch 5 ($n = 4$)[a]	Clutch 7 ($n = 4$)[a]	Clutch 8 ($n = 3$)[a]	S. MARIA – mean (SD)	Clutch 6 ($n = 2$)[a]	Clutch 9 ($n = 4$)[a]	P. REAL – mean (SD)
Egg contents												
As	<0.4	<0.4	1.9	0.94	1.4 (0.7)	0.86	<0.4	<0.4	0.86 (–)	<0.4	1.5	1.5 (–)
Cd	6.0	4.6	15	9.0	8.7 (4.6)	8.6	6.1	8.5	8 (1)	17	9.3	13 (5)
Cu	660	629	699	567	639 (56)	678	706	584	656 (64)	576	690	633 (81)
Pb	<1.2	12	9.3	35	19 (14)	4.1	<1.2	6.2	5.2 (1.5)	16	20	18 (3)
Zn	11920	10100	12950	12750	11930 (1299)	10720	12470	10480	11223 (1086)	11030	11820	11425 (559)
Eggshells												
As	95	89	40	66	73 (25)	135	55	49	80 (48)	47	62	55 (11)
Cd	11	8.7	10	235	66 (113)	120	40	86	82 (40)	9.4	6.5	8 (2)
Cu	3764	2579	3866	2938	3287 (629)	2683	4361	3380	3475 (843)	1487	1807	1647 (226)
Pb	185	217	1454	1005	715 (622)	408	251	175	278 (119)	211	176	194 (25)
Zn	7269	13290	6235	8144	8735 (3136)	7977	5605	7029	6870 (1194)	10350	4878	7614 (3869)

Mean and standard deviation corresponding to the tree different areas investigated.
[a] Number of samples pooled for each nest.

in chameleon egg contents than in egg contents of slider turtles (Burger and Gibbons, 1998), diamondback terrapins (Burger, 2002) and water snakes (Burger et al., 2005), but Cd and Pb concentrations in chameleon eggshells were higher than in slider turtle eggshells (Burger and Gibbons, 1998); in fact the ratio between Pb concentrations in contents and shells was different in the two species. This could be due to differences in the excretion pathways of chameleons and turtles.

4. Conclusions

A considerable decrease in Pb concentrations was observed in common chameleon egg samples collected in 2001 as compared with those analysed in 1997, which could be associated with a general decrease in the presence of this metal, especially in petrols. On the contrary, PCB levels in the present study were higher than in a previous sampling campaign in the same area. DDT concentrations did not vary from 1997 to 2001. Although data on contaminant concentrations in reptile eggs are scarce in the literature, what information there is suggests that pollution levels found in common chameleon eggs from Southwest Spain are generally lower than in eggs of other reptile species. We would note nonetheless that most of the data in the literature are from highly polluted areas.

Of the heavy metals and arsenic, concentrations of all the elements investigated, except Zn, were higher in chameleon eggshells than in eggs contents.

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